DRUG DISCOVERY

Edward A. Sausville, M.D., Ph.D.
Professor of Medicine
Associate Director for Clinical Research
Marlene & Stewart Greenebaum Cancer Center
University of Maryland at Baltimore

March 19, 2009

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Leads for Transition to Early Trials

DRUG DISCOVERY:
WHERE HAS IT WORKED?

Majority of Drug Targets:	% Top Sales
G-Protein Coupled Receptors	18
Nuclear (Hormone) Receptors	10
Ion Channels	16
Enzymes	approximately 50

Problem:

How to choose target likely to succeed
especially if directed at new target
(e.g. protein-protein interactions)?

Nature 384 suppl 11:5, 1996

DRUG DISCOVERY:
A SUCCESSION OF STYLES

Antiquity to 1960s:

Mixtures of natural products vs. bioassays
(e.g., digitalis, rauwolfia, penicillins, anthracyclines,
vinca, taxol, camptothecins)

1930s to present:

Pure compounds vs. bioassays
(e.g., sulfas, diuretics, hypoglycemics, antiHBP)

1960s to present:

Pure compounds vs. pure enzymes
(e.g., ACE inhibitors, cholesterol-lowering statins,
RT and protease inhibitors)

1980s to present:

Combinatorial methods to bring mixtures of compounds
vs. many targets

WHY COMPOUNDS
FAIL AND SLOW DOWN IN DEVELOPMENT

Reasons for failure	**Reasons for slowdown**
Toxicity, 22%	Synthetic complexity
Lack of efficacy, 31%	Low potency
Market reasons, 6%	Ambiguous toxicity finding
Poor biopharmaceutical properties, 41%	Inherently time-intensive target indication
	Poor biopharmaceutical properties

Modern Drug Discovery
January/February 1999
Modern Drug Discovery, **1999**, 2 *(1)*, 55-60.

TRADITIONAL PHARMACEUTICAL R&D
Suffers High Attrition*

Diagram illustrating the flow from initial candidate compound screening (10^3 - 10^5 compounds per screen) through "hits" and "leads" (100 leads), lead optimization, pre-clinical development (12 drug candidates) and clinical development (4-5 drug candidates) that results in only one NDA filing.

** Tufts CSDD, H&Q 1998; The Pfizer Journal, 1/2000*

TRADITIONAL PHARMACEUTICAL R&D
Costly* and Time Consuming**

Lead Discovery Research	Drug Development					
6 years	**8.9 Years**					
$230m	+71m	+56m	+169m	+169m	+44m}	**$608m***
Target ID Synthesis/ Screening Target Validation Lead Optimization	Preclinical	Ph1	Ph2	Ph3	Filed	

* *Lehman Brothers, 1997; ** Tufts CSDD*

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for Transition to Early Trials

TWO CONTRASTING DRUG-DISCOVERY "PHILOSOPHIES"

"EMPIRICAL": Recognize initial drug lead
by functionally useful effect
E.g.: penicillin (anti-bacterial effect)
rauwolfia (anti-hypertensive)
taxol (anti-tumor)
digoxin (cardiotonic / antiarrythmic)

"RATIONAL": Recognize drug by design or screen
against biochemical target's function
E.g.: HIV-protease inhibitor (anti-infection)
metoprolol (anti-hypertensive)
methotrexate (anti-tumor)

"EMPIRICAL" DRUG DISCOVERY

SCREEN   Pharmacology
↓   ⁄   ↓
BIOLOGICAL ACTIVITY   →   Chemistry
(in vitro/in vivo)

↓

OPTIMIZED SCHEDULE (*in vivo)*

↓

IND-DIRECTED TOX/FORMULATION

↓

PHASE I: DOSE/SCHEDULE HUMAN PHARM/TOX

↓

PHASE II: ACTIVITY

↓

PHASE III: COMPARE WITH STANDARD

PROBLEMS WITH EMPIRICAL MODELS

Lead optimization difficult without known biochemical target--How to optimize?

Value of screen depend on predictive value of screening model with biology of disease
E.g.: acid hypo-secretion or H2 receptor binding assay HIGHLY correlate with useful anti-ulcer Rx

Counter E.g.: anti-tumor activity in > 33% mouse models of cancer have at best 50% chance of >1 P2 trial for non=targeted cancer Rx's

Divorced from mechanism: an intriguing lead must be "deconvoluted"

KRN5500

Chemical Structure (prodrug outside cell)

Cell Membrane

Chemical Structure (prodrug inside cell)

Deacylation ↓ SAN-Gly

Chemical Structure (active metabolite)

⊥

Protein Synthesis (blocked)

EFFECT OF KRN5500 ON COLO-205 ATHYMIC MOUSE XENOGRAFTS

Line chart showing median tumor Weight (mg) in mice versus time (day posttumor Implantation). A dose-response relationship is shown for KRN5500 inhibition of tumor growth.

KRN5500 PLASMA CONCENTRATIONS ON EFFECTIVE SCHEDULE(20 MG/KG/D) IN MICE

Line chart showing plasma concentration (μM) in mice by Time (days) from 0 to 5 days. The plasma concentration in the mice goes up and down (peaks and troughs) quite a bit over time but overall it increases somewhat from the level on day 1. In addition, on days 3 and 4 peaks and trough levels are higher.

SUMMARY OF KRN-5500 PHASE I

26 patients as IV once per day over 5 days

Dose limiting toxicity = interstitial pneumonitis

MTD = 2.9 mg/M2/d x 5

Achieve only 0.75 - 1 μM at 3.7 mg/M2/d x 5

4/6 patients with >25% incr Cmax have

Data of J. P. Eder, DFCI

“RATIONAL” DRUG DISCOVERY

Flow chart showing steps from molecular target screen including Biochemical, Engineered cell, and Animal (yeast/worm/fish) to pharmacology (to affect target) to chemistry and then proceeds to the following steps in the order shown:

Target-dependent in vivo model
IND directed tox/form
Phase I: Dose/Schedule: human pharm/Tox;
?Affect Target
Phase II: Activity = ? affect target
Phase III. Compare with standard; stratify by target?

bcr-abl AS TARGET: RATIONALE

Apparently pathogenetic in t9:Q22 (Ph+) CML/ALL

Absence in normal tissues

Modulate signal transduction events downstream
- Maintenance of chronic phase
- Adjunct to bone marrow transplantation

bcr-abl FUSION PROTEIN

Schematic representation of regions in fusion protein.

McWhirter JR, EMBO *12:1533, 1993*

EXAMPLE OF "RATIONAL" APPROACH:
bcr-abl directed agents

Natural product empiric lead	Chemical structure of erbstatin , lavendustin and piceatannol
1st generation Synthetic	Chemical structure for AG957 and AG1112
2nd generation synthetic; in clinic	Chcmical structure of CGP 57148B = ST1571

STI571: An oral in vivo bcr-abl kinase inhibitor

Graphic illustration of antitumor activity in vivo in KU812 and U937 mice.

le Coutre et al, JNCI 91:163, 1999

EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

BRIAN J.DRUKER,M.D.,MOSHE TALPAZ,M.D.,DEBRA J.RESTA,R.N.,BIN PENG,PH.D.,
ELISABETH BUCHDUNGER,PH.D.,JOHN M.FORD,M.D.,NICHOLAS B.LYDON,PH.D.,HAGOP KANTARJIAN,M.D.,
RENAUD CAPDEVILLE,M.D.,SAYURI OHNO-JONES,B.S.,AND CHARLES L.SAWYERS,M.D.

Line chart showing white blood cells (cells x 10^{-3} /mm^3) over duration of treatment (days) with STI571. There is a significant drop in white cell count from day 1 by or before 30 days of the study. That lower white cell count continues through the period followed of approximately 100 - 140 days.

The second graph shows a decline in percent in metaphase for Ph chromosome t cells.

NEJM 344: 1031, 2001

MOLECULAR TARGET DEFINITION - HOW TO?

BIOLOGY
* Cytogenetics → Breakpoints → Molecules (bcr-abl)
* "Positive" selection from tumor DNA → Active oncogenes
  (signal transduction)
* Tumor gene expression profiling (CGAP)

"RETROFIT" ACTIVE MOLECULES:
* Binding partners (geldanamycin, rapamycin, fumagillin)
* Computational algorithm (molecule ↔ target)
  COMPARE
  Cluster analysis

"CLASSICAL:"
* Cell metabolism / Biochemistry
* Suggest single targets → Inefficient; Medicinal Chemistry possible

CHEMICAL GENETICS:
* Libraries of molecules and precisely defined organisms

Gene Expression: The Cell's Fingerprint

Bar chart comparing normal cells with cancer cells in Genes A through H expression. The bar chart shows that cancer cells out number normal cells in Genes A, C, E, and H. In H, few cells are normal and the vast majority are cancer cells. Normal cells out number cancer cells in Genes B, F, and G and with F there are significantly more normal cells. For Gene D the normal and cancer cells appear to be approximately equal.

Establishing for a cell the repertoire of genes expressed, together with the amount of gene products produced for each, yields a powerful "fingerprint". Comparing the fingerprints of a normal versus a cancer cell will highlight genes that by their suspicious absence or presence (such as Gene H) deserve further scientific scrutiny to determine whether such suspects play a role in cancer, or can be exploited in a test for early detection.

http://cgap.nci.nih.gov

At the bottom left of the slide is a logo from the National Cancer Institute - The Cancer Genome Anatomy Project.

This is information from a National Cancer Institute (NCI) document or website for the Cancer Genome Anatomy Project. It lists 5 different NIH ICs that are part of the CGAP initiatives

http://cgap.nci.nih.gov/

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

Graphic illustration of different survival probability based on gene expression in lymphoma cells.

Alizadeh et al, Nature 403: 503, 2000

GELDANAMYCIN: EXAMPLE OF BINDING PARTNER DEFINING TARGET

Chemical structure of benzoquinone (ansa ring and carbamate moieties)

	NSC	R
Geldanamycin	122750	OMe
17-AAG	330507	$NHCH_2CH=CH_2$

BENZOQUINOID ANSAMYCINS
INITIAL CELL PHARMACOLOGY – I

“Reverse” transformed phenotype of src-transformed
rat kidney cell line

decrease tyrosine phosphorylation of pp60src

not inhibit pp60 immune complex kinase directly but
these were inhibited from drug-treated cells

thus alter “intracellular environment” of src

(Uehara et al, MCB 6: 2198, 1986)

Decrease steady state phosphorylation levels
to 10% of control

decrease steady state level of pp60src by 30%

accelerate turnover of pp60src

(Uehara et al, Cancer Res 49: 780, 1989)

Diagram of ansamycin molecule linked to a bead by18 atom spacer.

GELDANAMYCIN BEADS
IDENTIFY HSP90 AS BINDING PARTNER

1) Bead-Geld

2) Bead-Geld + Geld

3) BeadGeld + Geldampicin

4) Bead

Neckers et al, PNAS 91:8324, 1994

Three graphic illustrations of the role of HSP 90 in cell function.

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for Transition to Early Trials

Diversity

Graphic illustration of 9 different snowflakes which vary widely one from another.

It is estimated that there are 10^{40} compounds in all of “chemical space”. Since the Big Bang, there have only been 10^{17} seconds.

- Peter Wipf

SOURCES OF DIVERSITY

"Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use

- "pure compound" collections
- extracts: aqueous/organic
- genetically altered producer organisms

Target non-selected chemical compound libraries

- peptide / protein
- non-peptide

Target-directed chemical compound libraries

- "classical" medicinal chemistry / bona fide crystal structure – derived
- "docked" lead structures into model

Natural Products: Unique arrays of the four “elements” which make a really useful drug

A circle is shown which is divided into four equal parts. Going clockwise from the top right segment they are labeled Base (-), Water (low dielectric), Acid (+), and Oil (high dielectric).

Sources of "Modern Drugs"

If one looks at the current drug scene from a chemical perspective (data from 1981 – 2002) then the following slides show reasonable approximations of the sources of drugs currently approved, World-wide, by the FDA or equivalent body.

Codes are:

N	**Natural Product**
ND	**Natural Product Derivative**
S*	**Natural Product Pharmacophore**
S	**Synthetic Compound**
B/V	**Biological / Vaccine**
(NM)	**Natural Product Mimic as a subdivision**

Sources of Drugs (1981-2002);
Extended Subdivisions n = 1031

A pie chart is shown and broken down as follows:

B = 12%
N = 5%
ND = 23%
S = 33%
S/NM = 10%
S* = 4%
S*/NM= 10%
V = 3%

Newman et al, J. Nat. Prod., 2003, 66, 1027-1037

EXAMPLES OF NP LEAD GENERATION OF NOVEL SCAFFOLDS

Guided by nature a compound library developed around nakijiquinones, which are natural inhibitors of the receptor tyrosine kinase called Her-2/Neu, produced analogs that inhibit two other receptor tyrosine kinases, VEGFR-3 and Tie-2.

Nature leads a library based on a natural product, Galanthamine, an antidemintia drug, turns up a new compound with a different activity. Secramine, a galanthamine-based molecule that blocks protein trafficking

Discovery of Lidocaine

***Central Asian camels refused to eat a certain type of reed**

***Characterization of gramine as the antifeedant principle led to the synthesis of isogramine**

***Taste-test: numbness; therefore, lead for anesthetic agent development**

Chemical structures of Gramine → Isogramine → Lidocaine

Courtesy of N. R. Farnsworth

Natural Product Isolation Tree

Flow chart illustration

“You are what you eat”

Journal of Natural Products, Nov. 1997;60 (11)

Dolabella auricularia
Dolastatins come from a *Symploca* species that they graze on

“Non-culturable” versus “Cultured” microbes

The microbial World has only just been scratched.
Much less than 1% of the available organisms have even been seen, let alone identified.

In soil, there are estimates of > 1000 species per gram
very few can be cultured

these may not be representative of the “Soil meta-Genome”

Over 1000 microbes per mL of seawater can be seen and only approximately 1% can be cultured using current methods.

SOURCES OF DIVERSITY

"Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use

- "pure compound" collections
- extracts: aqueous/organic
- genetically altered producer organisms

Target non-selected chemical compound libraries

- peptide / protein
- non-peptide

Target-directed chemical compound libraries

- "classical" medicinal chemistry / bona fide crystal structure – derived
- "docked" lead structures into model

TRIPEPTIDE COMBINATORIAL LIBRARY

XXX

Four amino acids in each position
$4^3 = 64$

A = Alanine
R = Arginine
T = Threonine
W = Tryptophan

after R. Houghten, 1999

NUMBER OF PEPTIDES
POSSIBLE WITH INCREASING LENGTH

Chart showing the length, peptide and number possible with increasing length, going from 400 possible peptides with 2 amino acids to over 25 billion with 8 amino acids

after R. Houghten, 1999

IC_{50} OF MIXTURES

A chart showing log concentration of a single active compound: IC_{50} = 1.0 nM, a single 1.0 nM active compound + 9 inactives: IC_{50} = 10 nM, and a single 1.0 nM active compound + 9,999 inactives: IC_{50} = 10,000 nM.

COMBINATORIAL LIBRARIES:
THE MIXTURE QUESTION

	Natural Product Extracts	Synthetic Combinatorial Mixtures
Direct screening of compound mixture	Yes	Yes
Discovery of highly active compounds	Yes	Yes
Equal concentrations of compounds	**No**	Yes
Chemical structure known	**No**	Yes
Synthetic pathway known	**No**	Yes
Structure – activity relationship known	**No**	Yes

after R. Houghten, 1999

NON-PEPTIDE "COMBINATORIAL" STRATEGIES COMBINE "SCAFFOLDS" (OR "BACKBONES") WITH "FUNCTIONAL GROUPS"

Graphic illustration and example of chemical structure

The Chemical Generation of Molecular Diversity from
http://www.netsci.org/Science/Combichem/feature01.html

THE RULE OF FIVE

An awareness tool for discovery chemists:

Compounds with two or more of the following characteristics are flagged as likely to have poor oral absorption

More than 5 H-bond donors
Molecular weight >500
c log P > 5
Sum of N's and O's (a rough measure of H-bond acceptors) > 10

Modern Drug Discovery
January/February 1999
Modern Drug Discovery, **1999**, 2 *(1)*, 55-60.

COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES

Chemical structure and synthesis

1. $CSIm_2$
2. HF/anisole

R1 x R2 x R3 = 49 x 51 x 42 = 104,958 compounds

after R. Houghten, 1999

BIOASSAYS
(READY APPLICATION OF SOLUBLE LIBRARIES)

Soluble Acceptors
- antibodies
- enzymes

Membrane-bound Receptors
- tissue homogenate
- functional cell based

Microorganisms: Disruption of Function
- bacteria
- fungi
- virus

Differentiation
- stem cells

In Vivo

after R. Houghten, 1999

POSITIONAL SCANNING BICYCLIC GUANIDINE LIBRARY
(κ RECEPTOR)

1/percent bound forR_1 position, R_2 position, and R_3 position.

after R. Houghten, 1999

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for
Transition to Early Trials

" RATIONAL":

-***Structure based design***
Biochemical Screen
Target-driven
Cell-based Screen
"EMPIRICAL"
Bioassay of effect

NMR-BASED SCREENING

Screen “fragment” like molecules with “leadlike” properties (MW <300; ClogP ~1.5)

Characterize ***binding*** and portion of molecule to which they bind

Ligands with weak affinities can be defined (~KD = 5mM)

Lead to high affinity binders through iterative screening

Can label protein of interest with isotopes “sensitive” to ligand effects (e.g. N15) and utilize proton resonances of drug to simultaneously allow definition of ligand and receptor binding sites

Hajduk et al, J Med Chem 48: 2518, 2005

NMR AS MEANS OF DEFINING BINDING SITES

E.G., BLEOMYCIN BIMDING TO DNA

NMR recording

1H NMR spectra of bleomycin at 100-MHz resolution. Each spectrum is an average of 512 scans. With 6 mM bleomycin in D_2O at pD 8.4; 6 mM bleomycin and 3.5 mM calf thymus DNA in D_2O, pD 8.4.

Horwitz et al, Biochemistry 16: 3641, 1977

BUILDING A DRUG LEAD

Graphic illustration of target molecule, screening of compound libraries and selection of lead compounds.

Successive iterations “build” more potent K_d

AFFINITIES OF
SELECTED BIARYL COMPOUNDS FOR BCL-XL

Illustration of 20 chemical structures and their respective NMR K_d (μM)

Petros et al, J Med Chem 49: 656, 2006

SECTION FROM A ^{15}N HSQC SPECTRUM OF BCL-XL IN THE PRESENCE AND ABSENCE OF COMPOUND

Plot of ^{15}N ppm over ^{1}H ppm

alone (white)
2 mM biaryl acid **1** (cyan)
2 mM biaryl acid **1** and 5 mM naphthol derivative **11** (pink)

Petros et al, J Med Chem 49: 656, 2006

SUPERPOSITION OF SEVEN LOW-ENERGY STRUCTURES CALCULATED FOR BCL-XL COMPLEXED TO 1 AND 11

Molecular model

Petros et al, J Med Chem 49: 656, 2006

THREE DIMENSIONAL VIEW OF GELDANAMYCIN BINDING POCKET IN AMINO TERMINUS OF HSP90

Molecular model

Stebbins et al, Cell 89:239, 1997

17-AAG BINDS TO HSP90 & SHARES IMPORTANT BIOLOGIC ACTIVITIES WITH GELDANAMYCIN

Two graphs. One shows erbB2 (% of base line) for 17-AAG GA over dose (nM). The other chart shows Raf-1 (% of base line) for 17-AAG GA over dose (nM).

Schulte & Neckers, Cancer Chemother Pharmacol 42: 273, 1998

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for
Transition to Early Trials

" RATIONAL":
- Structure based design
- *Biochemical Screen*
- Target-driven

Cell-based Screen
- "EMPIRICAL"
- Bioassay of effect

Cell cycle regulation by Cdc25 phosphatases

Graphic illustration

Regulation of Cell Cycle Progression by Cdc25: Cdk Activation

Graphic illustration

CDC25 Phosphatases and Cancer

CDC25A and B overexpressed in many cultured cancer cell lines.

Cdc25A suppresses apoptosis.

Overexpression of CDC25A or B has been detected in human breast, head and neck, cervical, skin, lymph, lung and gastric cancers.

Human CDC25A & B cooperated with Ha-RasG12V and CDC25A cooperated with Rb -/- in the oncogenic focus transformation of mouse embryonic fibroblasts and tumor formation in nude mice. Thus, Cdc25A & B may be human oncogenes.

Method for identifying Cdc25 phosphatase inhibitors

Graphic illustration of GST-Cdc25 in assay buffer with fluorescein diphosphate. There is another graphic illustration showing what happens after incubating one hour/RT. It becomes Read product (fluorescein monophosphate) on cytoflour II.

Chemical Screening Approach

Targeted Array Libraries

Diverse Chemical Libraries

Chemical structures showing lead compounds K_1 K_2 and K_3 and 13 analog structures for screening (including pBQ)

Compound 5 inhibits Cdc25

Chemical structure

Percent inhibition over log [Compound 5] M for Cdc25A, VHR, and PTP1B

$Cdc25B_2$ K_i approximately 2 μM

MALDI-TOF ANALYSES
Compound 5 binds tightly to the catalytic domain of Cdc25A

Two graphs, one shows the % intensity of m/z for DMSO.

The other shows % intensity for m/z for Compound 5.

Lixia PU

Compound Validation

Cellular: Cell Cycle

Biochemical: Substrate phosphorylation

Genetic: Chemical complementation

tsFT210 Cell System

Graphic illustration with functional and nonfunctional Cdk1.

Compound 5 causes G2/M arrest

Graphic illustrations

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for
Transition to Early Trials

" RATIONAL":

Structure based design
Biochemical Screen
Target-driven

Cell-based Screen
"EMPIRICAL"
Bioassay of effect

C/EBPα AS A TARGET FOR DEVELOPMENT OF NOVEL CANCER THERAPEUTICS

The transcription factor C/EBPα plays key roles in regulation of differentation of various cell lineages (adipocytes, keratinocytes, etc.)

Mutations in CEBPA (the gene coding for C/EBPα) are associated with development of AML [t(8;21) - subtypes M1 and M2]

CEBPA knock-out mice show no mature neutrophils

Conditional expression of CEBPA is sufficient to trigger neutrophilic differentiation

Pharmacologic modulators of CEBPA could act as differentiation inducers and thus limit proliferation of AML cells

CEBP Reporter Construct*

Graphic illustration (CEBP, TK, luciferase)

*Host cell for this construct is U-937

CEBPA Assay Timeline

Graphic illustration of assay procedure

*Sister plates processed for Alamar blue toxicity assay

C/EBPa Training Set: 1st Run compared to 2nd Run % Induction
Correlation Coefficient = .9265

Scatter plot shows % induction 2nd run compared with % induction 1st Run.

C/EB_a Training Set 1 uM Results*

% Alamar Fluorescence over % of control induction (relative to .625 uM approximately 100%)

*Data averaged from two independent assays

C/EBPa Screen: % Concentration Response Graphs

% induction (relative to .625 uM retinoic acid induction) for seven select compounds.

% of control induction over concentration

Categories of Confirmed Actives in CEPBα HTS

β-adrenergic agonists

Toxic compounds (stress signaling)

Retinoids

HDAC Inhibitors

Novel Drug Lead - Sterol mesylate

C/EBPa Frequency of Fold Induction for OSR Compounds in HTS

(Total of 135640 compounds tested)

Histogram showing frequently over fold induction

NSC67657 when screened AT 10μM scored 1.4 fold induction relative to RA control.

C/EBPa % Induction Dose Response Curves

Line chart showing % induction (test-cells only)/(Ret – Cells Only)* 100 by concentration of test compounds

Chemical structures for rimiterol HBr, Epinephrine, and DL-Isoproterenol HC1

NSC 67657, a novel sterol mesylate inducer of CEBPα with potential anti-leukemic activity

Chemical structure of NSC67657

Basis for Interest

Identified in a DTP high-throughput screen of > 140,000 compounds

Induced CEBP-luciferase activity at low concentrations: 50% activation at 40 nM

Induced differentiation in U937cells as measured by CD11b or CD11c antigens or NBT staining

Induced morphologic differentiation in HL60 cells

Induced cell surface markers of monocytic differentiation in AML patient blasts ex vivo

Secondary testing of NSC67657 in C/EBPa (U937) cells

Scatter charts indicating the following:

Dose-dependent increase of luciferase reporter activity (max. 1.6 fold)

Based on control induction of retinoic acid (1μM)

Activity occurs at non-toxic concentrations

Evidence for Morphologic Differentiation in HL60 Cells

Untreated control - largely myeloblasts

1 mM ATRA – Reduced cell numbers, segmented and cells resembling neutrophils

20 mM NSC 67657 – Reduced cell numbers, segmented and cells resembling neutrophils

GENERATION OF SAR AROUND STEROID MESYLATE LEAD

Related compounds available from the DTP Repository were tested in concentration-response format

No compounds with comparable activity were found (most were completely inactive)

Three compounds which showed some activity provided an initial SAR model

SAR data and chemical structures for NSC 67657
138980
260627
622259

Hierarchical cluster of 51 genes dysregulated >3 fold over control by NSC 67657 in HL60 cells.

Heat map of gene activation.

When compared to ATRA treated cells, several genes of the monocyte/ macrophage lineage were uniquely up regulated by NSC 67657.

NSC 67657 induces differentiation in different cell lines compared to ATRA

HL60 cells:
Can differentiate to either granulocytes or monocyte/macrophages

NB4 cells:
Can only differentiate into granulocytes

ATRA induces differentiation (measured by NBT reduction after 7 days) in both HL60 and NB4 cell lines, while NSC 67657 induced differentiation only in HL60 cells. This supports the monocyte/ macrophage lineage specific differentiation proposed from the gene expression studies

NSC 67657 induces a different pattern of cell surface markers compared to ATRA

Four graphs. Two show NB4 cells over CD14 and CD18. The other shows HL60 cells over CD14 and CD 18.

NSC 67657 induced CD14 expression only in HL60, not NB4 cells.
ATRA does not induce CD14 expression in either cell line (5 day incubation).

INITIAL STRUCTURE-ACTIVITY MODEL

Chemical structure of steroid ring and substitutions at R_2, R_3, R_6, R_{12} and R_{23}.

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for
Transition to Early Trials

" RATIONAL":
Structure based design
Biochemical Screen
Target-driven
Cell-based Screen
"EMPIRICAL"
Bioassay of effect

NCI IN VITRO DRUG SCREEN

1985 Hypothesis:
- Cell type specific agents
- Activity in solid tumors

Emerging Realities:
- Unique patterns of activity, cut across cell types
- and
- Cell type selective patterns found
- Correlations of compound activity
  - relate to molecular "target" expression
  - generate hypothesis re: molecular target

NCI IN VITRO CANCER CELL LINE SCREEN

60 cell lines
(8 breast, 2 prostate, 8 renal, 6 ovary, 7 colon,
6 brain, 9 lung, 8 melanoma, 6 hematopoietic)

48 hr exposure; protein stain O.D.

Graphic illustration

O.D. over time showing the following:

- Control
- "GI_{50}" = 50% inhibit
- "TGI" = 100% inhibit
- "LC_{50}" = 50% kill

National Cancer Institute Developmental Therapeutics Program Dose Response Curves

NSC: 643248-Q/2 ***(a rapamycin)*** **Exp. ID: 9503SC35-46**

Line chart showing all cell lines by percentage growth over Log_{10} of sample concentration (Molar). There is a significant drop at -6 molar of all cell lines that continues until approximately -5 and then in most cases levels off by -4 molar.

PATTERN RECOGNITION ALGORITHM: COMPARE

Goal: COMPARE degree of similarity of a new compound to standard agents

Calculate mean GI_{50}, TGI or LC_{50}

Display behavior of particular cell line (resistant or sensitive) as deflection from mean

Calculate Pearson correlation coefficient:
1 = identity; 0= no correlation

AGENTS WITH SIMILAR MECHANISMS HAVE SIMILAR MEAN GRAPHS

Leukemia

NSCLC

Small Cell Lung

Colon

CNS

Melanoma

Ovarian

Renal

All of the above over Taxol, Halichondrin B, and Daunorubicin

↓ ↓ ↓

Tubulin Topoisomerase II

THE COMPARE ALGORITHM
Seed: Rubidazone

164011	1.000	Rubidazone
82151	0.921	Daunomycin
123127	0.915	Adriamycin
665934	0.891	Epipodophyllotoxin analogue
Discreet	0.880	Gyrase-To-TOPO analogue
Discreet	0.867	AMSA analogue
267469	0.865	Deoxydoxorubicin
305884	0.865	Acodazole HCL
665935	0.864	Epipodophyllotoxin analogue
668380	0.861	Azatoxin analogue
639659	0.854	Adriamycin analogue
644946	0.850	Epipodophyllotoxin analogue
254681	0.848	Daunomycin analogue
Discreet	0.847	Epipodophyllotoxin analogue
Discreet	0.843	Epipodophyllotoxin analogue
180510	0.842	Daunomycin analogue
Discreet	0.837	Epipodophyllotoxin analogue
Discreet	0.833	Gyrase-To-TOPO analogue

RELATIVE EGF RECEPTOR mRNA EXPRESSION

Relative expression for breast, prostate, renal, ovarian, melanoma, CNS, colon, NSCLC, Leukemia.

COMPARE ANALYSIS:
EGF RECEPTOR

RANK	CORRELATION	CHEMICAL NAME
1	0.71	TGFα-PE40
2	0.66	Toxin-Δ53L, MW=43K
7	0.57	EGFR Tyrosine Kinase Inhibitor
88	0.43	EGFR Tyrosine Kinase Inhibitor

40,421 COMPOUNDS IN THE NCI DATABASE

DRUG TARGET CLUSTERINGS
REVEAL CLUES TO MECHANISM

Heat map.

Drugs (clustered) over genes (clustered)

5FU/DPYD L-Asparaginase/ASNS

Nature Genetics 24: 236, 2000; http://dtp.nci.nih.gov

OUTLINE OF PRESENTATION

General Introduction

Definition of Drug Targets

Generating Diversity

Definition of Lead Structures

Qualifying Lead for Transition to Early Trials

GOALS OF PRECLINICAL DRUG STUDIES

Regulatory framework

IND = "Investigational New Drug" application = approval by FDA to conduct human studies; main criterion : SAFETY AND LIKELY REVERSIBLE TOXICITY = allows *start* of Phase I trials

NDA = "New Drug Application" = basis for sale to public; main criteria: SAFETY AND SOME MEASURE OF EFFICACY = *result* of Phase II/III trials

COMPONENTS OF AN IND

The goal of the pre-clinical process

"Form 1571"	Pharmacology/Toxicology
Table of Contents	
Intro Statement / Plan	Prior human experience
Investigator Brochure	Additional info – data monitoring, Quality assurance
Clinical Protocol	
Chemistry, Manufacture, Control	

OBJECTIVES OF PRECLINICAL PHARMACOLOGY STUDIES FOR ANTI-NEOPLASTIC DRUGS

Development of Sensitive Analytical Methods for Drugs in Biological Fluids & tissues

Determine *In Vitro* Stability and Protein Binding

Determine Pharmacokinetics in Rodents (& Dogs)

Identification and Analysis of Metabolites

Define Optimal Dose Schedule and Blood Sampling Times

Define C_P and/or AUC with Efficacy, Safety & Toxicity

Analog Evaluation - Determine Optimal Development Candidate

OBJECTIVES OF PRECLINICAL TOXICOLOGY STUDIES

DETERMINE IN APPROPRIATE ANIMAL MODELS:

The Maximum Tolerated Dose (MTD)

Dose Limiting Toxicities (DLT)

Schedule-Dependent Toxicity

Reversibility of Adverse Effects

A Safe Clinical Starting Dose

FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS: ONCOLOGY Rx

DRUGS

- Two Species - Rodent & Non-rodent
- Clinical Route & Schedule
  - Follow NCI Guidelines
- Pharmacokinetics - Optional

BIOLOGICALS

- Most Relevant Species
- Clinical Route & Schedule

CORRELATION BETWEEN 20S PROTEASOME INHIBITORY POTENCY & GROWTH INHIBITION FOR 13 DIPEPTIDE BORONIC ACIDS

Chemical structure and plot of Mean FI_{50} (nM) over K_j (nM)

Adams et al, Cancer Res 59:2615, 1999

EFFECT OF PS-341
ON PC-3 TUMOR GROWTH IN MICE

Plot of tumor volume (% vehicle) over Week (1 through 6) when treatment is administered at week 1.

Vehicle
(n=15)

PS-341
0.3 mg/kg
(n=15)

PS-341
1.0 mg/kg
(n=10)

Adams et al, Cancer Res 59:2615, 1999

EFFECT OF PS-341
ON 20S PROTEASOME ACTIVITY

Two bar charts. One shows 20S activity (% vehicle) for mouse WBC over PS-341 (mg/kg). The other one shows 20S activity (% vehicle) over PS-341 (mg/kg).

Adams et al, Cancer Res 59:2615, 1999

PS-341: INTERSPECIES

Q: Is the 'safe' dose in animals in the efficacy range for man?

Species	Dose (mg/kg)	Dose (mg/m^2)	% 20S Proteasome Inhibition
Mouse	1.0	3.0	80
Rat	0.25	1.5	80
NHP	0.067	0.8	70

***In white blood cells at 1.0 h, post-dose**

Ref: Adams, *et al, Cancer Res* <u>59</u>:2615, 1999

Ex Vivo Proteasome Activity:
1 Hour Post Treatment

Scatter chart showing % 20S activity over PS-341 (Log dose, mg/m^2)

ACKNOWLEDGEMENTS

NCI

J. Tomaszewski
M. Alley
M. Hollingshead / S. Stinson
J. Johnson
A. Monks / N. Scudiero
S. Bates
D. Zaharevitz / R. Gussio
S. Decker
R. Shoemaker / M. Currens

J. Adams
Millenium
J. Lazo
U. Pittsburgh